Three-Dimentional Histometry of Bile Ducts in the Porta Hepatis Tissue in Cases of Biliary Atresia

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Summary

In order to determine the optimal level of transection at the porta hepatis in patients with biliary atresia, the sizes and distributions of bile ducts at several levels of the resected porta hepatis tissues were investigated.

Specimens were obtained from 12 cases of noncorrectable biliary atresia. Five micron serial sections were available in 6 cases. Only macroserial sections were available in the other 6 cases. Histometric studies were carried out on sections at 250 μ intervals in each case using a newly developed color image analyzer.

Measurements were made of the area, circumference, major and minor axes of all the bile ducts as well as the section area, the area of the connective tissue and the liver tissue. Bile ducts were classified into three groups according to their areas: small, medium and large.

At the most proximal level of section, small and medium-sized ducts were almost exclusively encounterd. The total area increased rapidly between 0.25–1.5 mm distal from the most proximal levels. The levels of rapid increase in area corresponded to the levels where the connective tissue was 90% of the whole section (the 90% levels). The bile ducts decreased rapidly in size and in number at the levels 1.0–1.5 mm distal from the 90% levels (the levels of histological atresia). The total areas were almost constant at the levels between the 90% levels and the levels of histological atresia in some cases, but were variable at each level in the others. Comparison of three-dimentional reconstructions using microcomputer with the histometric study revealed that these levels of variable area corresponded to disruptions of the bile ducts. The maximum total area levels and the levels where the largest bile ducts were observed corresponded to the maximum connective tissue levels. The small and medium sized ducts were noticed at all levels of section. The large ducts were restrictedly observed at the levels distal from the 90% levels.

These results indicate the possibility both of histological atresias being located very near the liver and of disruptions of the bile ducts at any level in the connective tissue.

From this study, it was confirmed that the main aim of transection of the porta hepatis is the entire removal of the connective tissue.

Key words: Biliary atresia, Bile Ducts, Porta hepatis, Histometry, Reconstruction using Microcomputer.
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Introduction

The distribution pattern of the tiny bile ducts in the porta hepatis tissue of patients with biliary atresia has been repeatedly discussed in relation to the method of transection of the porta hepatis and to the postoperative bile flow. This subject has been investigated both through three-dimentional reconstructions of the tiny bile ducts and through comparative studies between the histometry of the tiny bile ducts and the prognosis of the patients.

In this issue, a histometric study of the tiny bile ducts at several levels of the porta hepatis tissue using a newly developed color image analyzer are reported. The sizes, shapes and distributions of the tiny bile ducts at each level of section are investigated and the level of transection of the porta hepatis is discussed.

Terminology

PORTA HEPATIS TISSUE indicates the resected specimen of the porta hepatis per se. SECTION OF THE PORTA HEPATIS indicates each section obtained through a serial slice of the porta hepatis tissue. CONNECTIVE TISSUE and LIVER TISSUE are the two main parts in the section of the porta hepatis. LEVEL OF THE SECTION is the term indicating the level in the porta hepatis tissue. It is actually expressed by the serial number of the section of the porta hepatis. LIVER SIDE or PROXIMAL SIDE of the porta hepatis tissue are the term used to indicate the upward direction in the tissue. THE DISTAL SIDE indicates the downward direction. THE MOST PROXIMAL LEVEL OF SECTION refers to the section which is located at the top of the porta hepatis tissue with the section area of more than 5 mm^2. TINY BILE DUCTS refers to the ductal structures in the porta hepatis tissue. MAJOR AXIS of the bile ducts refers to the longest dimension. MINOR AXIS refers to the shortest dimension. This study is called THREE DIMENTIONAL HISTOMETRY.

Patients

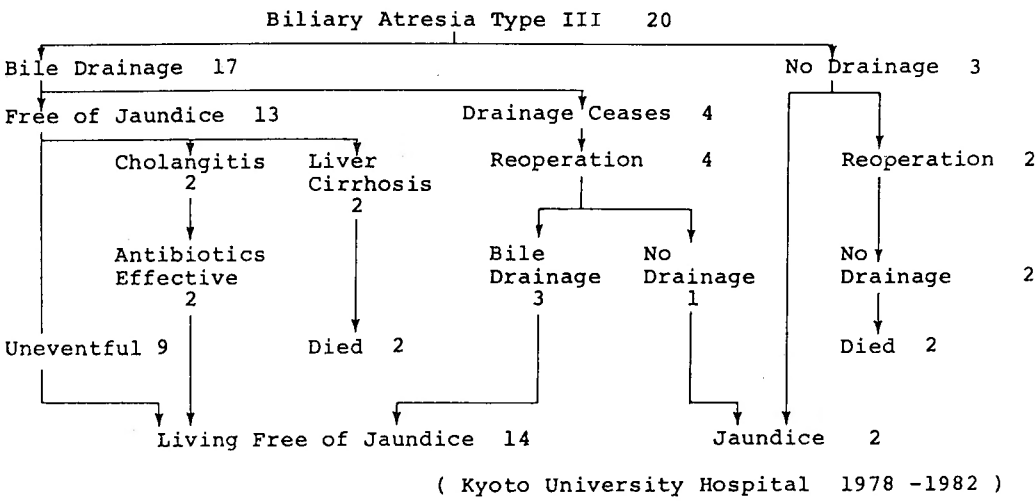
Twenty cases of noncorrectable biliary atresia were treated at our institute during the period from January 1978, to December 1982 by a procedure of jejunal interposition hepatic portoduodenostomy with a intestinal valve[43]. Overall results are shown in Figure 1. Twelve of these cases were available for this study. Clinical courses of them are summarized in Table 1.

Serial sections were obtained from 6 of these cases but were not available in the other 6 cases as the specimens had already been examined before the beginning of this study. Only macroserial sections were available in those cases.

Methods

Porta hepatis tissues were obtained from 12 cases with noncorrectable biliary atresia, and were fixed in 10% formalin for 5 days and then embedded in paraffin. Five micron serial sections were made through the upper 3–5 mm of the specimen using an automatic slicer at room

Figure 1. Overall results of 20 cases of biliary atresia treated by jejunal interposition hepatic portoduodenostomy with an intestinal valve in the last 5 years.



temperature. The sections were then stained with hematoxiline and eosin, elastica van Gieson and trichrome. Histometric studies were carried out on sections at 250 μ intervals, i.e. on every 50th section. Nine to 13 sections were investigated per case.

An OLYMPUS COLOR IMAGE ANALYZER VIP 21 CH was used for the histometry (Figure 2). Microscopic images were obtained in color on a display. The sizes, circumferences, major and minor axes of the objects on the display were automatically calculated by tracing their shapes. The values were shown on the display or were printed out. The images were clear enough to observe the fine pathology of the bile ducts.

Measurements were made of the section area, the area of the connective tissue and the area

Table 1. Clinical courses of 12 cases. Serial sections were obtained from Cases 1–6. Only macroserial sections were available in Cases 7–12.

Case	Sex	Onset of Jaundice (days)	Operation (days)	Type of Atresia	Postoperative Bile Flow	Clinical Courses	Final Follow-up
1.	m	40	67	IIIan	good	Reoperation, live free of jaundice	(1y 8m)
2.	f	0	41	IIIbn	no	Reoperation, died	(1y)
3.	f	0	68	IIIbn	good	Cholangitis, live free of jaundice	(1y 6m)
4.	m	14	77	IIIbn	good	Uneventful, live free of jaundice	(1y 7m)
5.	f	27	40	IIIan	good	Reoperation, live with jaundice	(1y 1m)
6.	f	5	23	IIIcn	no	live with jaundice	(10m)
7.	m	30	82	IIIan	good	Cirrhosis, died	(1y 1m)
8.	f	4	19	IIIan	good	Uneventful, live free of jaundice	(4y 5m)
9.	f	13	31	IIIcn	good	Uneventful, live free of jaundice	(4y 8m)
10.	m	30	71	IIIcn	good	Cirrhosis, died	(1y 8m)
11.	m	50	62	IIIan	good	Cholangitis, live free of jaundice	(3y 1m)
12.	f	2	20	IIIcn	good	Uneventful, live free of jaundice	(5y)

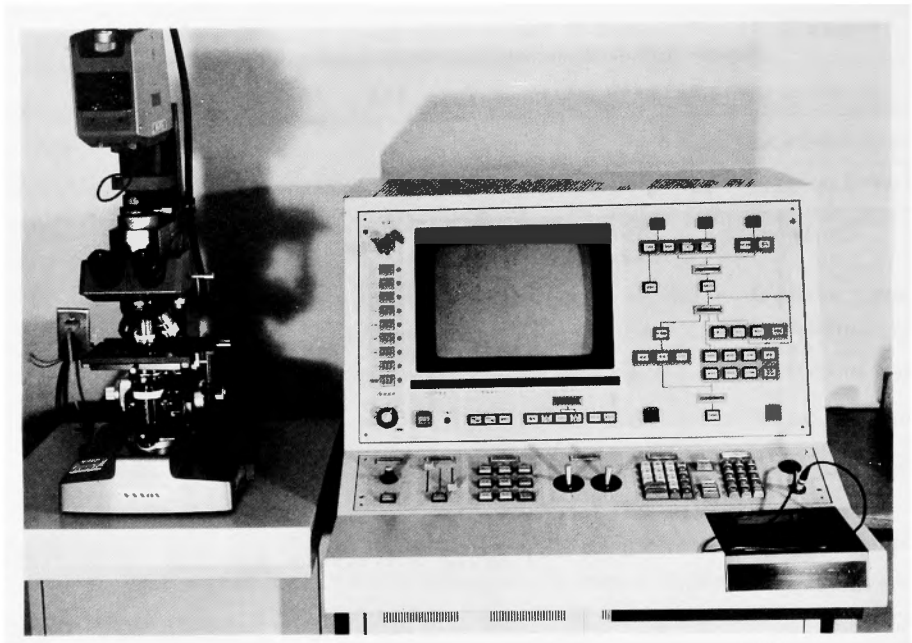


Figure 2. OLYMPUS COLOR IMAGE ANALYZER VIP 21CH.
(left: microscope, right: computer image analysing system)

of the liver tissue at each level of section at low magnification. The area, circumference, major and minor axes of each bile duct were then measured at 400× magnification. At this magnification, the COLOR IMAGE ANALYZER displayed, at one time, a 250×160 μ area of the section. The total section was traversed in order to observe all of the tiny bile ducts. All the ductal structures with columnar epithelium and cuboidal epithelium were picked out for measurement. Lumens without epithelium were not regarded as bile ducts. Partially epithelized

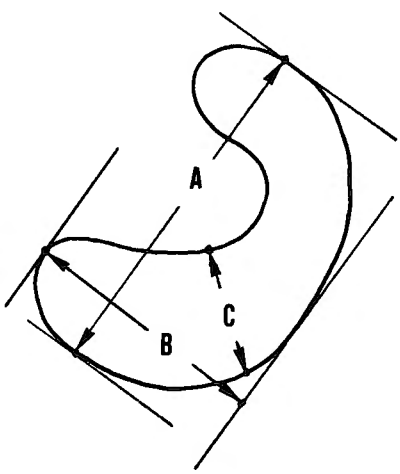


Figure 3. A: Major axis calculated by the analyzer.
B: Minor axis calculated by the analyzer.
C: Minor axis measured by the author.

lumens were, however, regarded as desquamized bile ducts.

The values of the areas, circumferences and major axes calculated by the ANALYZER were taken as their final values. However, the values of the minor axis determined by the ANALYZER were the distance B in Figure 3. Therefore, the values of the minor axes were determined by measuring the distance C. Data was stored on floppy-disks for later microcomputer analysis.

Tiny bile ducts were classified into three groups according to their areas:

Group 1: bile ducts with an area of less than 1,000 μ^2.

Group 2: bile ducts with an area between 1,000–5,000 μ^2.

Group 3: bile ducts with an area of more than 5,000 μ^2.

The total number and total area of all the tiny bile ducts as well as the total number and total area of the tiny bile ducts in each group were calculated at each section.

Results

1. The area of the whole section, the connective tissue and the liver tissue as well as the proportion of the connective tissue to the whole section. (Figures 4 and 5, Table 3)

The porta heaptis tissues in all cases had thin layers of liver tissue on the top of the specimen except in Case 3.

The maximum and minimum whole section areas were 47.03 mm^2 (Case 12), and 3.53 mm^2 (Case 5), respectively. The maximum and minimum areas of the connective tissues were 47.03 mm^2 (Case 12) and 0 mm^2 (Case 5), respectively.

Both connective tissue and liver tissue were found at the most proximal level. The proportion of connective tissue increased from the proximal level distally and eventually occupied the whole of the section. The changes from liver tissue to connective tissue were rapid in Cases 5 and 6, and gradual in Cases 1 and 4. Therefore, the proportion of connective tissue to the whole section at each level of the section differed from case to case.

2. Correlation between the area and the circumference, as well as the major and minor axes of the bile ducts. (Table 2, Figure 6)

There existed a definite positive correlation between the area of the bile ducts and the circumference. The area also correlated well with the major and minor axes of the bile ducts. The circumference was most closely correlated with the area.

3. The bile ducts at the most proximal level of the sections. (Figures 5 and 7)

Mean total area of the bile ducts at the most proximal level was $45,926 \pm 38,794$ μ^2 (N=12). The maximum value was 142,377 μ^2 and the minimum was 12,130 μ^2. Mean total number of the bile ducts at the most proximal level was 126.8 ± 115.0 (N=12). The maximum number was 414 and the minimum was 30.

Group 1 and 2 bile ducts were almost exclusively encountered at this level of section. There were few Group 3 bile ducts.

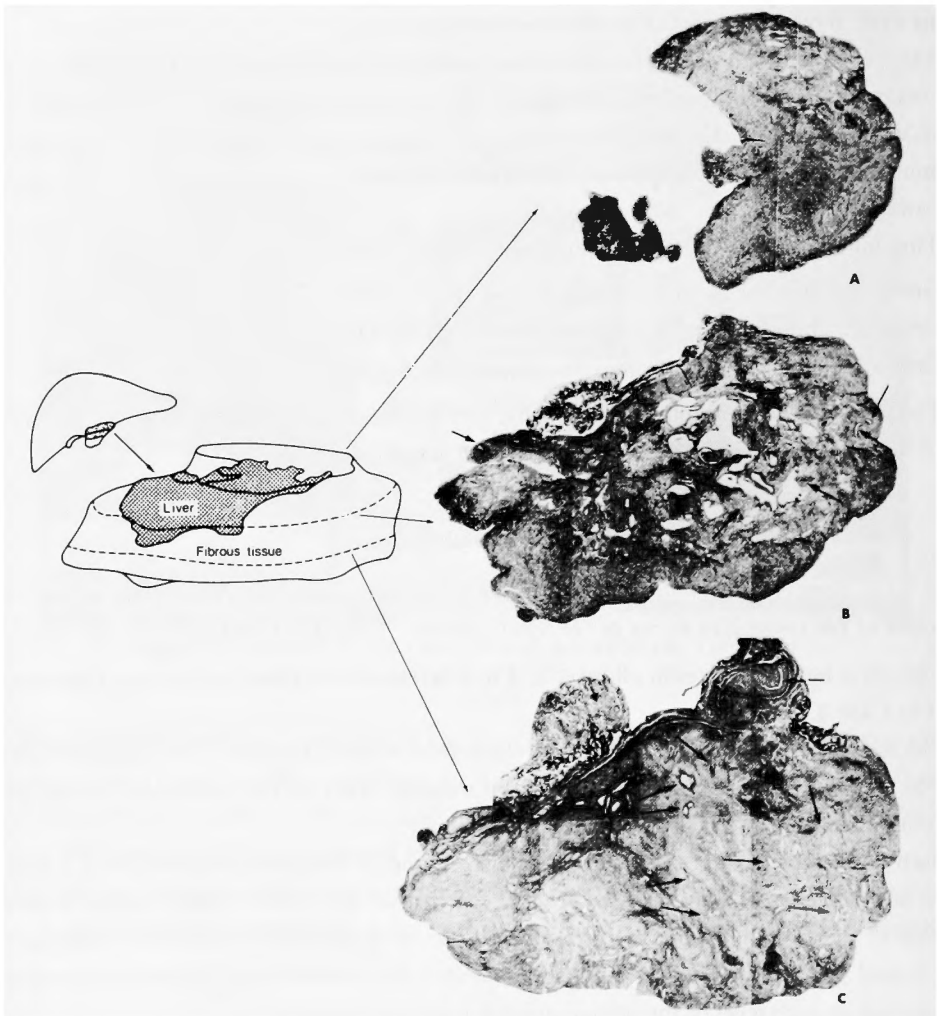


Figure 4. Three representative levels of section in Case 1.
A. Level 1: the most proximal level.
B. Level 4: the 90% connective tissue level.
C. Level 6: the maximum total area level.
Small arrows indiate Group 3 bile duts.

4. Total number and total area of the bile ducts at various levels of section in the porta hepatis tissue. (Table 3, Figure 8)

Total numbers and total areas of the tiny bile ducts in 12 cases are summarized in Table 3. Their distributions were investigated in the 6 cases in which serial sections were available (Figure 8). Three common patterns were recognized in the distributions.

The first pattern is as follows:

The total areas were small at the most proximal levels, then increased rapidly between 0.25 mm–1.5 mm distal from these levels. This was observed in all cases except in Case 3 in which the specimen studied had no liver tissue at the top. Interestingly, the areas increased at

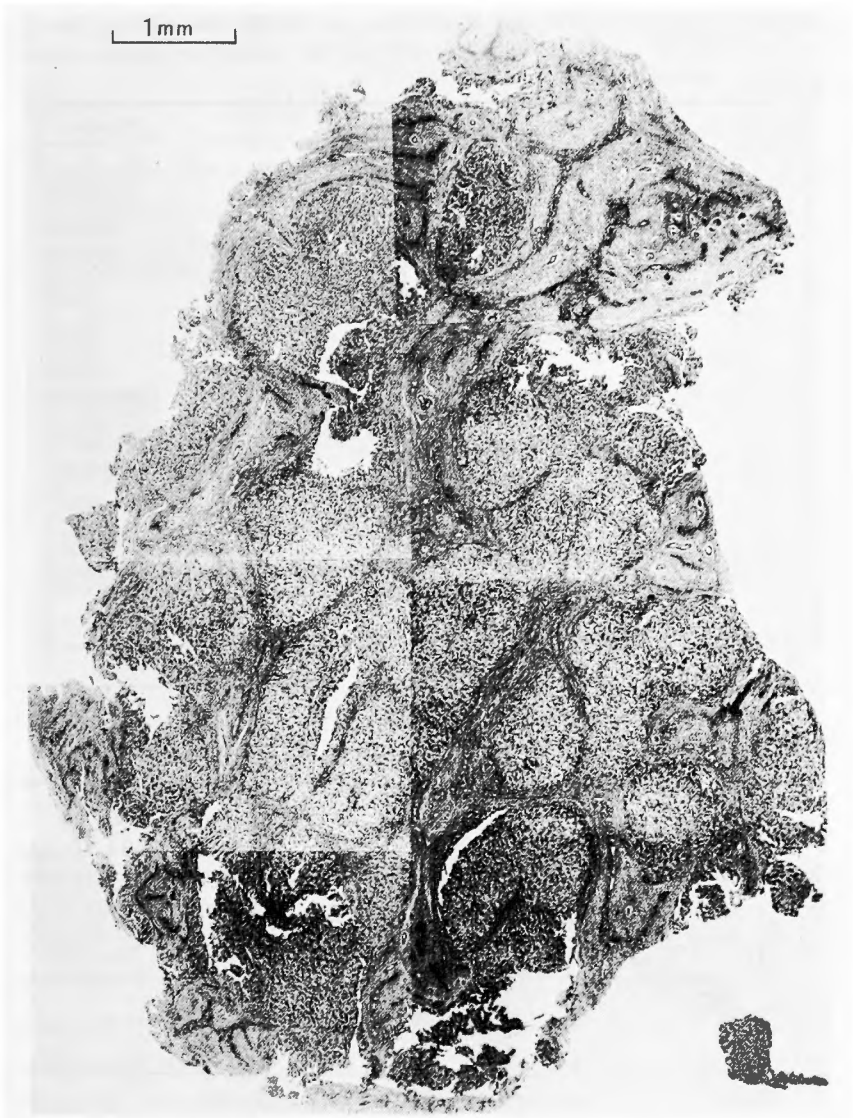


Figure 5. A microscopic view of the section at the most proximal level in Case 12. A mixture of connective and liver tissue was observed. The connective tissue area was 45% of the whole section area. The Group 1 and Group 2 ducts were almost exclusively noticed at this level of section.

the levels where the connective tissue was 90% of the whole section (90% level).

The second common pattern was that the total areas were almost constant about 1.0–1.5 mm distal from the 90% level. This was observed in Cases 1, 3 and 6. In Cases 2 and 4, the total areas varied at each level of section. The significance of this instability will be considered in Result 9.

The third common feature was that the level of histological atresia is located very near the liver. The tiny bile ducts decreased rapidly in size and in number, and disappeared at the 9th,

Table 2. Correlation coefficients between the area and the circumference as well as the major and minor axes of the bile ducts at the maximum total area levels in 12 cases.

Case	Level	Correlation Coefficients between the Area and the			Number of Bile Ducts
		Circumference	Major axis	Minor axis	
1	(6)	0.892	0.874	0.666	207
2	(5)	0.940	0.883	0.800	110
3	(5)	0.906	0.879	0.821	210
4	(7)	0.924	0.886	0.953	65
5	(10)	0.923	0.844	0.852	144
6	(6)	0.904	0.819	0.868	162
7	(b)	0.895	0.905	0.932	81
8	(b)	0.816	0.851	0.922	78
9	(b)	0.880	0.863	0.729	169
10	(b)	0.823	0.804	0.954	102
11	(b)	0.854	0.852	0.845	268
12	(b)	0.857	0.756	0.873	229

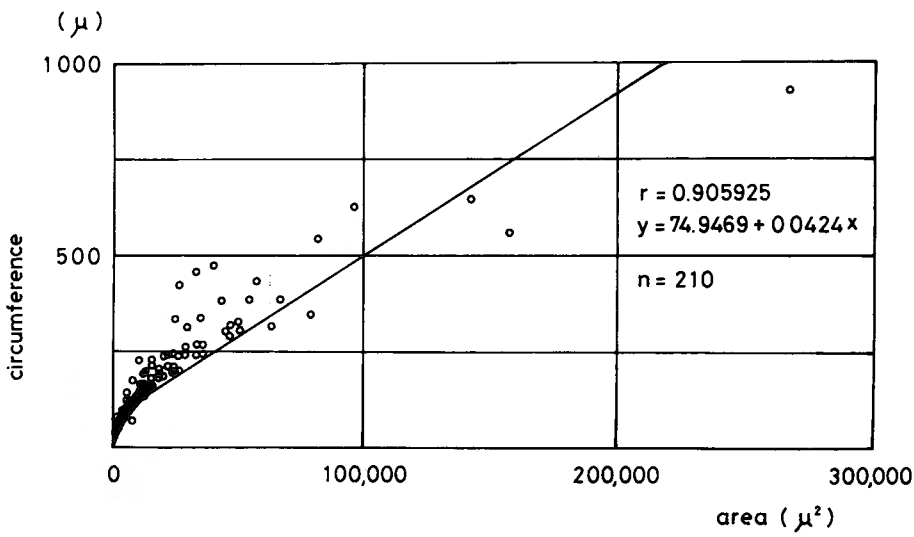


Figure 6. Correlation between area and circumference of bile ducts at level 5 in case 3.

9th and 12th levels in Cases 1, 3 and 6, respectively. This level was 1.0–1.5 mm distal from the 90% level in each case. In the other three cases, the areas and the numbers of the bile ducts were maintained to the most distal level studied.

5. The maximum values of the total number and total area of the bile duct, the area, circumference, major and minor axes of the largest bile duct and the maximum area of the connective tissue. (Table 4)

The maximum values and corresponding levels in the porta hepatis tissue are shown in Table 4.

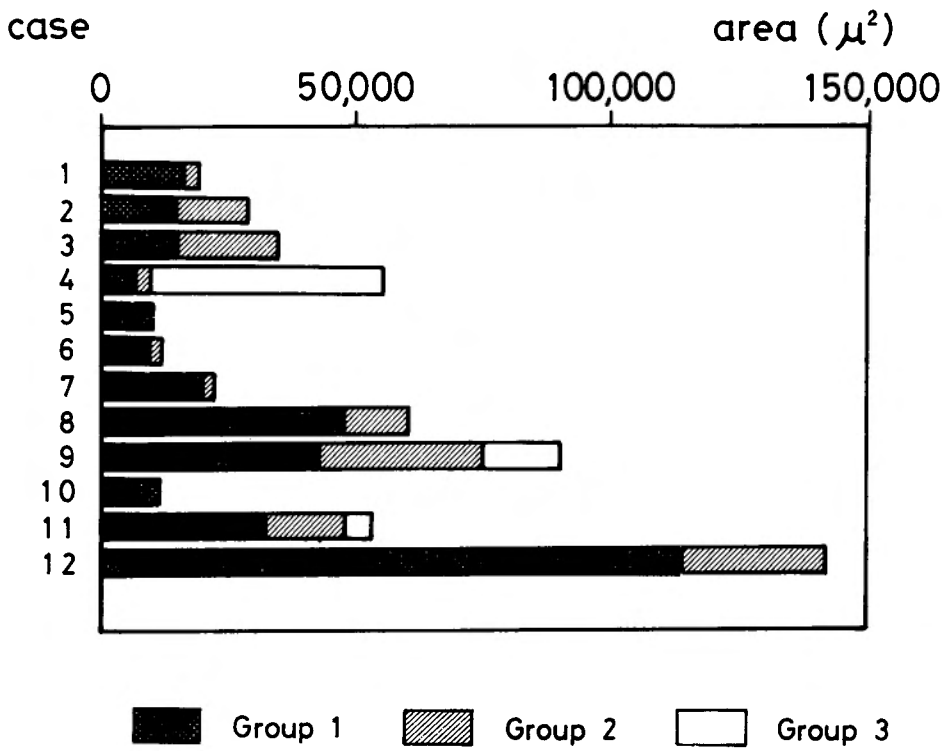


Figure 7. The Area of bile ducts at the most proximal levels in 12 cases.

The mean and standard deviation of the maximum total numbers of the bile ducts was 191.5±90.2 (79–414); the maximum total areas 308,455±213,689 μ^2 (90,559–855,862 μ^2); the maximum areas of the largest bile duct 170,480±238,113 μ^2 (14,500–401,400 μ^2); the maximum circumferences 3,232±1,829 μ (1,010–7,380 μ); the maximum major axes 1,008±514 μ (239–2240 μ); the maximum minor axes 187.7±96.1 μ (66.2–385 μ); the maximum connective tissue area 28.25±10.89 mm^2 (9.63–47.03 mm^2).

The levels of the maximum area of the bile ducts were located at the 8th, 5th, 7th, 7th, 12th and 6th levels, respectively. The levels of maximum connective tissue were situated at the 8th, 5th, 7th, 7th, 13th and 7th levels, respectively. The levels of maximum circumference and of the major and minor axes were also located at the maximum connective tissue levels except in Case 4. Thus the largest bile ducts were found at the maximum connective tissue area levels. The maximum total area levels were at or a little proximal to the maximum connective tissue area levels. The maximum total number levels were not always the same as the maximum total area levels.

6. The distribution of the Group 1 bile ducts. (Table 3, Figure 8)

Group 1 bile ducts were noticed at all levels. In the three cases with levels of histological atresia, Group 1 ducts decreased rapidly in size and number at those levels. Group 1 ducts were

Table 3. Total numbers and total areas of all the bile ducts and of the bile ducts in each group, the areas of the whole section, connective tissue and the liver tissue at each level. Levels are represented by 1-13 in Cases 1-6 (the distances between the nearest two levels are 0.25 mm) and by a-c in Cases 7-12.

Case	Level	Number				Area (μ^2)				Area of the Largest Duct (μ^2)	Area of the (mm^2)			C/S (%)
		Total	G1	G2	G3	Total	G1	G2	G3		Whole Section	Conn. Tissue	Liver	
1	1	59	56	2	1	24938	16608	2900	5430	5430	8.7	5.3	3.4	61
	2	39	38	1	0	12025	10585	1440	0	1440	14.7	11.0	3.7	75
	3	65	58	7	0	31723	20583	11140	0	2230	17.9	15.8	2.1	88
	4	147	118	24	5	149075	35265	55340	58470	20300	21.5	21.1	0.4	98
	5	144	124	16	4	181627	35847	35780	110000	63800	23.8	23.6	0.2	99
	6	207	171	27	9	213251	43531	65030	104690	22700	26.9	26.9	0.0	100
	7	109	83	19	7	164285	27085	40250	96950	24300	27.5	27.5	0.0	100
	8	49	37	5	7	174321	13601	11060	149660	64100	28.1	28.1	0.0	100
	9	15	13	0	2	36399	5129	0	31270	24600	25.3	25.3	0.0	100
	10	12	8	4	0	10599	1759	8840	0	3330	18.3	18.3	0.0	100
2	1	77	70	7	0	29319	14829	14490	0	4290	8.0	6.0	2.0	75
	2	66	56	7	3	68761	14921	15390	38450	22900	12.6	11.1	1.5	88
	3	121	105	15	1	73040	23300	41650	8090	8090	19.8	16.9	3.0	85
	4	98	77	17	4	109457	19527	35480	54450	27100	25.4	25.2	0.3	99
	5	110	90	13	7	260894	22952	28760	209182	117000	26.0	26.0	0.0	100
	6	104	84	14	6	146149	21559	27760	96830	29400	23.8	23.8	0.0	100
	7	96	76	18	2	76830	20430	39340	17060	10800	20.0	20.0	0.0	100
	8	66	55	8	3	109081	16741	15880	76460	38000	18.1	18.1	0.0	100
	9	48	38	6	4	46393	8598	10055	27740	8460	14.8	14.8	0.0	100
3	1	54	43	11	0	35265	15265	20000	0	2990	8.6	8.6	0.0	100
	2	87	60	21	6	141000	20482	41778	78740	25600	12.5	12.5	0.0	100
	3	132	95	29	8	173922	36972	54100	82850	19800	25.4	25.4	0.0	100
	4	168	111	47	10	250990	45180	101280	104530	27800	35.8	35.8	0.0	100
	5	210	138	58	14	301611	44971	125750	130890	26700	43.8	43.8	0.0	100
	6	217	167	43	7	274714	61044	80510	133160	30200	44.5	44.5	0.0	100
	7	142	95	38	9	298126	23476	87110	187540	120000	45.3	45.3	0.0	100
	8	69	51	15	3	74916	17526	36240	21150	8670	38.2	38.2	0.0	100
	9	28	19	6	3	42412	4972	13780	23660	11000	32.6	32.6	0.0	100
	10	7	5	2	0	7583	1273	6310	0	4440	25.8	25.8	0.0	100
4	1	30	26	2	2	55917	6727	3090	46100	28400	7.2	6.7	0.5	93
	2	34	26	4	4	207073	8673	10110	188290	145000	21.4	20.1	1.3	94
	3	79	71	5	3	52470	15430	7330	29710	13200	28.9	27.7	1.2	96
	4	31	22	5	4	118375	7045	14130	97200	37500	30.2	29.4	0.8	97
	5	33	25	3	5	102558	5808	5370	91380	35100	33.0	32.4	0.5	98
	6	60	48	6	6	149493	9243	17460	122790	42300	34.3	34.3	0.0	100
	7	61	48	8	5	140932	13912	17340	109680	36400	31.9	31.9	0.0	100
	8	65	52	7	6	221283	9013	16200	196070	147000	31.9	31.9	0.0	100
	9	52	42	4	6	102062	11602	8460	82000	33400	27.3	27.3	0.0	100
	10	43	35	3	5	82862	7422	4620	70820	23000	20.5	20.5	0.0	100
5	1	20	20	0	0	4063	4063	0	0	702	3.5	0.0	3.5	0
	2	58	58	0	0	10591	10591	0	0	639	6.1	0.0	6.1	0
	3	78	76	2	0	18043	14483	3560	0	2110	7.2	0.6	6.6	8
	4	139	122	16	1	71290	40100	25840	5350	5350	9.4	3.5	6.0	37
	5	50	41	9	0	33855	13665	20190	0	4640	10.3	8.3	2.0	81
	6	85	74	7	4	70958	22198	17150	31610	11900	9.7	9.7	0.0	100
	7	104	87	9	8	247519	26159	22190	199170	65300	9.7	9.7	0.0	100
	8	70	47	16	7	224029	16589	32260	175180	56100	10.9	10.9	0.0	100
	9	98	79	16	3	238738	28838	31250	178650	155500	12.4	12.4	0.0	100
	10	144	110	23	11	601989	31669	52130	518190	342000	15.0	15.0	0.0	100
	11	138	109	21	8	388694	36044	40430	312220	175000	19.2	19.2	0.0	100
	12	140	106	31	3	525206	36765	60141	428300	401400	21.3	21.3	0.0	100
	13	164	125	22	17	490312	49414	44008	396890	103000	29.0	29.0	0.0	100

6	1	37	35	2	0	12579	9707	2870	0	1710	13.4	2.4	11.0	18
	2	84	69	14	1	47087	20907	21010	5170	5170	18.4	7.2	11.2	39
	3	102	92	10	0	45016	27255	17761	0	2970	24.8	14.0	10.8	57
	4	152	142	10	0	62908	43538	19370	0	2950	26.5	15.8	10.7	60
	5	183	144	35	4	191564	50184	64950	76430	60300	31.3	29.1	2.2	93
	6	162	137	22	3	198784	46194	47870	104720	88300	33.0	32.4	0.5	98
	7	109	85	20	4	183294	30225	40059	113010	63700	34.9	34.9	0.0	100
	8	118	96	18	4	115538	39718	28330	47490	28500	31.5	31.5	0.0	100
	9	107	81	25	1	98019	31479	51440	15100	15100	28.9	28.9	0.0	100
	10	79	55	22	2	86413	21413	50470	14530	8680	28.4	28.4	0.0	100
	11	47	25	19	3	97494	8314	38230	50950	37900	26.7	26.7	0.0	100
	12	24	16	6	2	37656	6766	16250	14640	8180	25.5	25.5	0.0	100
	13	10	7	3	0	7429	1739	5690	0	3010	22.4	22.4	0.0	100
7	a	131	129	2	0	22703	20143	2560	0	1550	33.0	10.1	22.8	31
	b	81	49	22	10	348444	16194	52810	279440	78800	25.0	25.0	0.0	100
8	a	243	234	9	0	60981	48091	12890	0	2090	17.6	4.4	13.2	25
	b	78	55	13	10	163318	20168	22580	120570	19700	17.7	17.7	0.0	100
	c	2	0	1	1	36280	0	3280	33000	33000	6.6	6.6	0.0	100
9	a	87	85	2	0	26783	22263	4520	0	3320	3.6	1.4	2.2	39
	b	169	147	20	2	90560	42830	32030	15700	8410	13.5	9.6	3.9	71
	c	79	72	5	2	49402	21732	7600	20070	14500	10.9	9.0	1.9	82
10	a	94	94	0	0	12131	12131	0	0	394	12.3	5.5	6.8	45
	b	102	73	17	12	240576	19015	34841	186720	43400	18.8	18.8	0.0	100
	c	32	25	4	3	155138	8208	7030	139900	105000	22.1	22.1	0.0	100
11	a	237	232	4	1	53756	32536	15890	5330	5330	17.2	7.0	10.2	41
	b	268	231	28	9	204889	65949	52160	86780	22600	19.5	18.8	0.7	96
	c	158	138	17	3	115478	36648	35590	43240	21620	20.0	20.0	0.0	100
12	a	414	396	18	0	142377	114087	28290	0	2670	32.3	14.1	18.2	44
	b	229	160	47	22	853862	67642	98450	687770	362000	47.0	47.0	0.0	100
	c	138	88	38	12	338708	36787	85171	216750	50300	25.0	25.0	0.0	100

Table 4. The maixmum values of the following items and the corresponding levels: total number and total area of the bile ducts, the area, circumference, major and minor axes of the bile ducts and the connective tissue area. Levels are indicated in the parentheses.

Case	Maximum Total Number	Maximum Total Area (μ^3)	Maximum Area (μ^2)	Maximum Circumference (μ)	Maximum Major Axis (μ)	Maximum Minor Axis (μ)	Maximum Connective Tissue Area (mm^2)
1	207 (6)	213,251.0 (6)	64,100.0 (8)	2400 (5)	855 (8)	131 (8)	28.06 (8)
2	121 (3)	260,894.0 (5)	117,000.0 (5)	2940 (5)	772 (5)	141 (5)	26.04 (5)
3	217 (6)	301,611.0 (5)	120,000.0 (7)	2970 (7)	1150 (7)	134 (7)	45.34 (7)
4	79 (3)	221,283.0 (7)	147,000.0 (7)	4430 (2)	1240 (2)	153 (7)	34.27 (7)
5	164 (13)	601,989.0 (10)	401,400.0 (12)	7380 (12)	2240 (12)	288 (12)	28.96 (13)
6	183 (5)	198,784.0 (6)	88,300.0 (6)	4100 (7)	977 (7)	318 (4)	34.86 (7)
7	131 (a)	348,444.0 (b)	78,000.0 (b)	2170 (b)	745 (b)	194 (b)	24.96 (b)
8	243 (a)	163,318.0 (b)	33,000.0 (c)	1120 (b)	388 (b)	155 (c)	17.72 (b)
9	169 (b)	90,559.0 (b)	14,500.0 (c)	1010 (c)	239 (b)	66 (c)	9.63 (b)
10	102 (b)	240,576.0 (b)	105,000.0 (c)	2450 (c)	1030 (c)	200 (b)	22.11 (c)
11	268 (b)	204,889.0 (b)	22,600.0 (b)	2405 (c)	1021 (c)	87 (b)	19.98 (c)
12	414 (a)	855,862.0 (b)	854,862.0 (b)	5410 (b)	1440 (b)	385 (b)	47.03 (b)

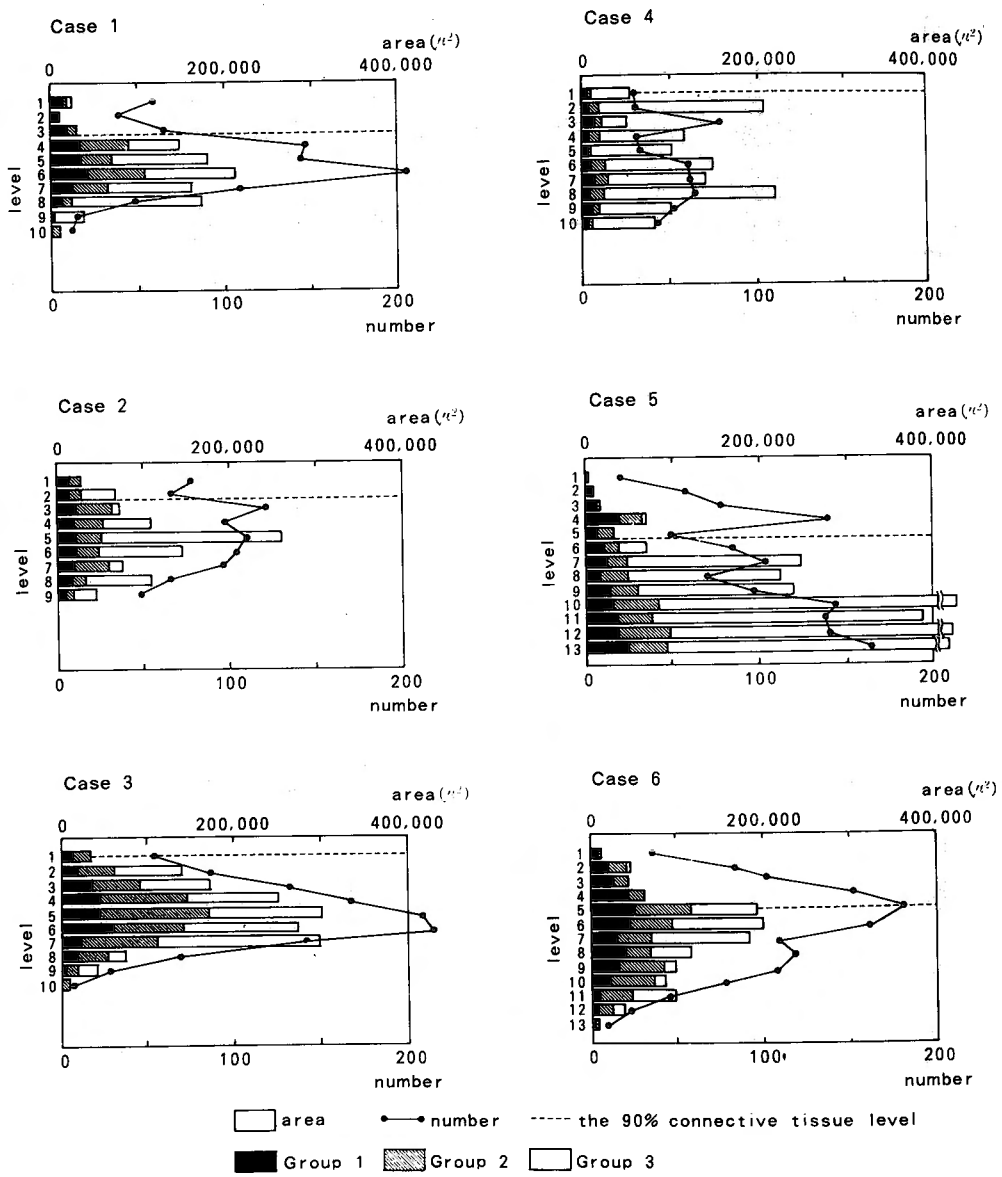


Figure 8. The change of area and number of bile ducts at several levels of section. Lines indicate the number. Bars indicate the area. Dotted lines show the 90% connective tissue levels.

present at all levels in the other three cases.

The total numbers of ducts depended to a large extent on the numbers of Group 1 ducts, as Group 1 ducts were far more numerous than those of Groups 2 and 3. From the proximal level distally, the total area of Group 1 ducts increased and then decreased again in Cases 1, 2, 3 and 6; increased continuously in Case 5; and had two peak levels in Case 4. The total areas of Group 1 ducts reached its peak at the maximum number levels of the Group 1 ducts.

7. The distribution of the Group 3 bile ducts (Table 3, Figure 8)

There were few Group 3 bile ducts at the most proximal levels. More than two Group 3 ducts were first noticed in each case at the 90% connective tissue levels in each case (the levels of a rapid increase in total area). Group 3 ducts disappeared at the levels of histological atresia in Cases 1, 3 and 6, but in the other three cases, the numbers of Group 3 ducts ranged from 4 to 17 at the most distal levels investigated.

As the area of the individual Group 3 bile ducts is much larger than those of Groups 1 and 2, the total areas of the bile ducts were mainly determined by the area of Group 3 ducts. The total areas of Group 3 ducts were almost unchanged through certain levels in the porta hepatis tissue in Cases 1, 3, 5 and partially in 6. They were variable at each level in Cases 2, 4, and partially in 6.

8. The distribution of the Group 2 ducts (Table 3, Figure 8)

The numbers and areas of the Group 2 ducts were midway between those of the Groups 1 and 3. Group 2 ducts were observed even at the most proximal levels. The numbers and areas of Group 2 ducts increased, remained constant then decreased in Cases 1, 2, 3 and 6; increased constantly in Case 5; and remained unchanged in Case 4.

9. Comparisons of three-dimensional reconstructions with histometric results (Figures 9 and 10)

Three-dimentional reconstructions of Group 3 bile ducts with their areas and numbers at several levels of section are depicted in Cases 4 and 6. Levels where the areas remained un-

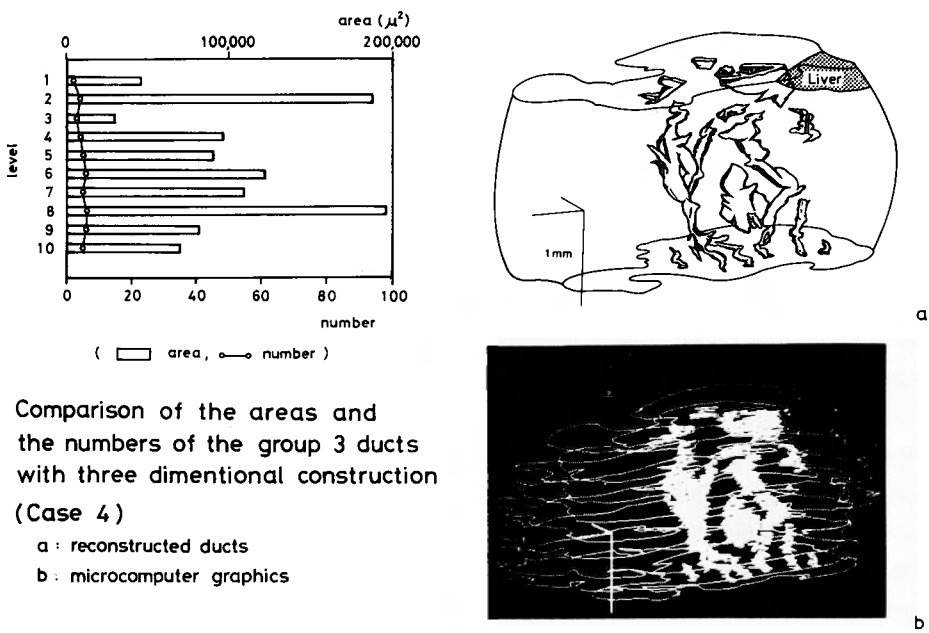


Figure 9. Comparison of the areas and numbers of Group 3 ducts with three-dimentional reconstruction. (Case 4)

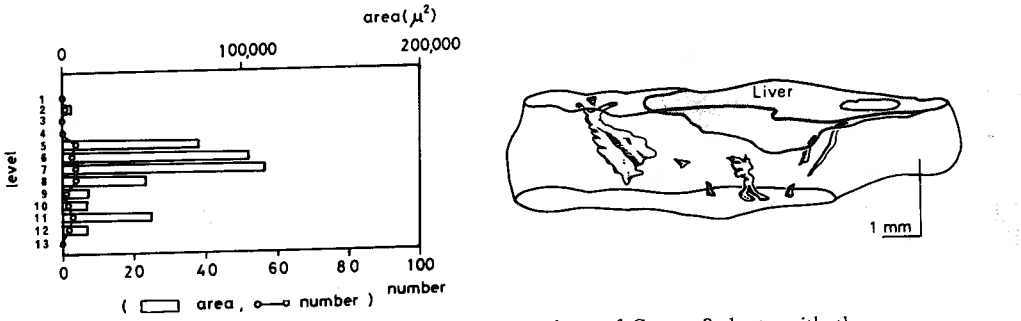


Figure 10. Comparison of the areas and numbers of Group 3 ducts with three-dimentional reconstruction. (Case 6)

changed coincided with the levels where the ducts were patent. Levels of rapid increase in area of the ducts were the levels where the ducts appeared in the tissue. Similarly, levels of rapid decrease in area were the levels of disappearance of the ducts. This study confirmed that instability of the area at each level of section in histometry is a sign of disruption of the ducts.

Discussion

Since the beginning of hepatic portal dissection on patients with biliary atresia by Dr. Kasai[17] in 1957, the existence of numbers of tiny bile ducts in the porta hepatis tissue[18] has been widely recognized. A large amount of research has been focused on the tiny bile ducts in connection with the operative procedure and the etiology of biliary atresia.

There are three main trends in the study of the tiny bile ducts in porta hepatis tissue; histometric studies[3,4,7,8,14,23,27-31,33], three-dimentional reconstructions[5,34], and histological studies[9,10,44]. Three-dimentional constructions have provided us with some information about the patency and the connections of the bile ducts in the porta hepatis tissue. Histometric studies are employed to investigate the relationship between the size of the bile ducts and the postoperative bile flow.

Numerous histometric studies have been available in the world literature since the first report of Kasai[18]. However, no conclusion has been reached yet. Kasai[18] said that good excretion of bile was obtained in cases which had bile ducts with a diameter of more than 200 μ. Miyano[28], Chandra[4], Hitch[14] and Ohi[33] reported that a good bile drainage was obtained in cases where large bile ducts were present. On the other hand, Gautier[8], Mustard[30], Lawrence[23], Nishiura[31], Miyano[29] and Matsuo[27] obtained no correlation between the size of the bile ducts and the postoperative bile flow. Initially, the size of the ducts was specified only by their diameters. With the development of microcomputer image analyzing systems, it has become easy to measure the area and circumference in addition to the diameter of the ducts. However, from the results, it can be seen that there is no great difference between using the areas, circumferences or the major and minor axes as the variable specifying the sizes. Nishiura[31] and Matsuo[27] stressed the importance of the small-sized tiny bile ducts in bile drainage, reporting a close correlation between the total area of the tiny bile ducts and the postoperative bile flow.

Miyano[29], however, found no evidence to support this.

One reason why a definite conclusion to this approach has not been obtained is that changes in the liver as well as the morphology of the bile ducts in the porta hepatis influence the bile drainage. Many issues are available which deal with the relationship between the postoperative bile flow and liver fibrosis, proliferation of the bile ducts and the degeneration of liver cells[6,18,25,32,36,38-40].

The other reason is that these studies have offered no information about the structures of the bile ducts in the porta hepatis. As shown in this paper, the number and area of the tiny bile ducts varied from level to level in the porta hepatis tissue. So, the total features of the bile ducts can not be reasonably estimated from one section. Miyano[29] reported the mean and standard deviation of the numbers of the tiny bile ducts was 34 ± 24 and that of the areas was $34{,}400 \pm 23{,}900\ \mu$ in 13 good bile flow cases. These values are much smaller than our results. However, the criteria for the level of dissection of the porta hepatis differs among institutes, as is discussed later. Therefore, it is unreasonable to compare values from different institutes as the results were obtained at different levels. It is of the great importance to establish a standard level for investigation.

At the most proximal level of section, a mixture of liver and connective tissue is noticed[31]. As shown in the results, the connective tissue increased from the proximal level distally and eventually occupied the whole of the section. The change from the liver to the connective tissue was rapid in some case and more gradual in the others. The porportion of the connective tissue area to the whole section area in each case can be used to express the proximity to the liver. It cannot, however, be used to compare absolute levels between cases.

In spite of this disadvantage, histometry is beneficial in determining the kinds of bile ducts which are functional in bile drainage, as it enables us to observe all the ducts in the section. The OLYPMUS COLOR IMAGE ANALYZER is suitable for this purpose. Even at high magnification, it produces an image clear enough to check all the small bile ducts and degenerated ducts.

The second approach, the reconstruction study, is important in understanding the structures of the bile ducts, especially the patency of them in terms of operative procedures. Only two reports[5,34] are available in the literature. Chiba[5] proved the connection of ducts in the porta hepatis tissue with the lobular bile ducts in the liver through an investigation of 9 autopsy cases. Okamoto[34] showed disrupted tiny bile ducts through the study of porta hepatis specimens obtained at operation.

The disadvantage of reconstruction studies is the difficulty in representing all the tiny bile ducts in the tissue. As shown in the results, numerous tiny bile ducts ranging from 0 to more than $400{,}000\ \mu^2$ were distributed in a $20\ mm^2$ section. Therefore, it is impossible to make a perfect representation of the ducts. The author presented reconstructions of the bile ducts using microcomputer graphics[26]. It is a simpler method than wax reconstruction reported by Hanai[12] and Ohi[32]. However, it is still impossible to obtain a complete representation of the bile ducts. For reasons of simplicity, the author made reconstructions only of Group 3 ducts

neglecting smaller ones. The figures demonstrated by Okamoto[34] are similar to those in this issue.

The third approach, morphological studies, on several levels of section have been reported by some investigators[9,11,44]. Tuchiya[44] compared the tiny bile ducts in the porta hepatis with those of the hepatic duct, common duct and gallbladder, and pointed out that tiny bile ducts less than 100 μ in diameter were usually found at the porta hepatis level. Gautier[9] made a comparison of the bile ducts in the connective tissue at the porta hepatis, an intermediate level and the junction of the hepatic and common duct, and claimed that few ducts were found at the latter zone. Haas[11] reported morphological similarities between the bile ducts in the liver and those at porta hepatis as well as those at the extrahepatic level. The author's present investigation has focused on the serial morphological changes of the tiny bile ducts in the vicinity of transition from liver to connective tissue, the so-called "porta hepatis" described in their reports.

Three-dimentional histometry reported in this paper has both the advantages of previously reported histometry and of reconstruction studies. This study was intended to evaluate a three-dimentional figure of *all* the tiny bile ducts through measuring their areas at several levels of section. The numbers of cases in the present study is too small to evaluate the relationship between the bile duct morphology and postoperative bile flow. Only the pattern of distribution of the ducts is reported.

There were common patterns in the distributions of the bile ducts.

Levels of rapid increase in the area of the bile ducts were noticed, and corresponded to the 90% connective tissue levels.

The level of histological atresia lied very near the liver. Tuchiya[44] reported that histological atresia is usually noticed at the level of the hepatic duct. Gautier[9] showed that the atresia is observed at the junction of the hepatic and the cystic duct. In this study, however, three of the cases revealed the atresias being located at the level 1.0–1.5 mm from the liver. These findings are important to evaluate the etiology as well as the operative procedures of biliary atresia.

The existence of various sized bile ducts in the porta hepatis tissue is clear from previous histometric studies[3,4,7,8,14,23,27–31,33]. However, the distributions of ducts of each size had not been investigated.

Numerous small-sized tiny bile ducts were found at the most proximal level of section. The ducts at this level are morphologically similar to the proliferating bile ductules in the portal area of the liver. Few large-sized tiny bile ducts were observed. In some institutes, the dissection is carried higher into the liver to meet the patent ducts in cases where no ductal structures are identified by frozen section[2,41] or no bile flow is observed at the plane of dissection[15]. The present study revealed that no large ducts are encountered by higher dissection into the liver. The functional importance of the small ducts at this level, however, is still unclear. The total area of small-sized ducts was unchanged through all levels of section. Gautier[8] said that periductal glands are normally present at the porta hepatis, citing textbooks from the 19th

century[37]. Okamoto[34] reconstructed a figure of clusters of small-sized ducts opening into a larger duct via a connecting duct. Ohi[33] insisted that all the small-sized ducts with a diameter of 75–190 μ are glands and are not functional in bile drainage. However, the author observed small-sized tiny bile ducts both in, and not in, clusters. Thus, the author cannot agree with Ohi's opinion entirely. The author considered that the small-sized tiny bile ducts consist of proliferating bile ducts, glands and ductal structures of unknown origin.

The large-sized ducts appeared in the porta hepatis tissue at the level of 90% connective tissue. The largest bile duct was found at the level of maximum area of the connective tissue. Kimura's belief[20] that the largest bile duct is located in the vicinity of the liver parenchyma proved to be true from the presented results. As the total area of the ducts in section depends on the area of the large-sized ducts, the maximum total area was noticed at about this level. These findings are highly suggestive of an etiological relationship between the large-sized ducts and the connective tissue in the porta hepatis.

In some cases, the area was unchanged through all levels from the 90% level to the level of atresia, but varied in the other cases. Reconstruction revealed disrupted bile ducts in the latter cases at the levels very near the liver.

The criteria for transection of the porta hepatis differs among institutes[13].

The left and right margins of transection are determined by different anatomical landmarks in each institute: the points of the hepatic artery joining the liver[1,19], the junction of teres hepatis and the left branch of the portal vein for the left margin and gallbladder fossa for the right margin[35], the first branches of the hepatic arteries[16] or the branches of the portal vein[7]. As to the depth of transection into the liver, Kasai[19] stressed the level of the posterior margin of the portal vein. Practically, however, the depth is judged by the liver tissue attached to the top of the resected tissue. Some authors[1,42] recommended the transection should not to be extended into the liver parenchyma. Others[15,35,21] insist that the transection should be made a little deeper into the liver. Abscess is reported to develop at the portoenterostomy[22] and the postoperative results are reported to be poor when transection is made 1–2 cm deep into the liver[20]. At the author's institute, thin liver parenchyma attached to the resected specimen has been regarded as the sign of an entire resection of the connective tissue. It was possible to observe the morphological changes of the bile ducts at the transitional zone from the liver to the connective tissue using our specimens.

From the results of the study, the author comfirmes the main aim of transection of porta hepatis is the entire removal of the connective tissue. One reason is the possibility of histological atresia being located very near the liver. The second is a possibility of disruption of the bile ducts at any level in the connective tissue. The author showed cases in which all the bile ducts disappeared 1–2 mm distal from the 90% connective tissue level. It is essential in these cases to transect the porta hepatis at a level, at least, within 1 mm distal from the liver attachment. However, as it is not possible to measure the thickness of the residual connective tissue, entire removal is the safer procedure. Okamoto[34] showed a reconstruction of disrupted large-sized ducts in the porta hepatis tissue. Our results indicate that the

levels of disruption of the large ducts can be located at any level of section. Suruga[41] and Lilly[24] determined the level of transection by examining the large ducts in frozen section of the stump of a specimen taken at operation. However, the size alone does not guarantee the patency of a ducts. The entire removal of the connective tissue is essential in order to make a transection beyond the points of disruption. Recently some investigators[7,16] reported a 2–3 cm wide transection extending laterally from the porta hepatis. This seems reasonable for the purpose of the entire removal of the connective tissue.

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和文抄録

三次元的組織計測による胆道閉鎖症肝門部微小胆管の形態学的検討

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胆道閉鎖症における肝門部切離の理想的なレベルを探求する目的で，最新の画像処理装置を使用した組織計測を肝門部切除標本の連続切片に対して行った．

対象は，12例のⅢ型胆道閉鎖症患児の手術時採取した肝門部標本である．うち6例は5μの連続切片を，他の6例は粗大連続切片を利用した．標本には，ヘマトキシリン・エオジン染色，弾性線維染色，トリクロム染色を施した．OLYMPUS COLOR IMAGE ANALYZER VIP 21 CH を使用し，最も肝臓側の切片から250μごとの切片で，標本面積，結合織面積，肝組織面積，およびすべての胆管の面積，周囲長，長径，短径の計測を行った．胆管は，大，中，小の3群に分類した．

胆管の分布に，次のようなパタンを認めた．胆管総面積，総数は各レベルによって大きく変化した．最も肝臓寄りの切片では，中小の胆管のみで，大きい胆管はほとんど認めなかった．総面積は最も肝臓側より0.25-1.5 mm 末梢で急増した．このレベルは結合織面積が全標本面積の90%を占めるレベル(以下90%レベル)に一致した．3例ではこの90% レベルより更に末梢1.0-1.5 mm 以内で，胆管面積・数とも激減し，0に近づいた．同部が組織学的な胆道閉鎖部位と思われた．90%レベルと組織学的胆道閉鎖部位の間のレベルで，ほぼ胆管総面積が一定であった症例と，大きく変動した症例があった．マイクロコンピューターを使った立体構築との比較により，レベルによる面積の大きい変動は胆管の断裂の所見であることを確認した．胆管総面積が最大となるレベル及び最も大きい胆管が存在するレベルは，結合織面積が最大のレベルに一致した．中小の胆管の分布は，大きい胆管の分布と異なっていた．前者はすべてのレベルで認められ，後者は90%レベルより末梢にのみ認められた．

以上の如く，症例によっては，肝実質から1 mm 以内の近傍に組織学的な胆道閉鎖部位が存在すること，さらに胆管の断裂があらゆるレベルに存在する可能性のあることが確認された．これらの点から，胆道閉鎖症の肝門部切離にあたっては，結合織の完全な切除が手術のポイントであると結論した．